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**Development of a novel ion-pairing UPLC method with cation-exchange solid-phase extraction for determination of free timolol in human plasma.**

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**Public Summary:**

**Scientific Abstract:**

A novel UPLC-UV method was developed for analysis of timolol in human plasma using a simple, fast, and cost effective ion-exchange SPE procedure, followed by separation on a C18 UPLC column with a mobile phase consisting of acetonitrile, phosphate buffer, and sodium 1-octane sulfonate as an ion pairing agent. The method was fully validated according to US-FDA guidelines, and was found to be sufficiently accurate and precise for analysis of timolol in human plasma for clinical pharmacokinetic studies. The application of ion-exchange SPE cartridges for purification of timolol in plasma produced excellent percent recoveries and good sample clean-up, while the ion-pairing separation described here allowed quantitation of timolol without interference from endogenous sample components. The method lower limit of detection was 1.7ng/mL and the lower limit of quantitation was 5.0ng/mL, allowing for analysis of therapeutic concentrations of timolol in plasma.

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